



# Stabilized Synthetic Nucleic Acids

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**NCCLS** “The analytical performance and quality control needed for a testing process must satisfy the medical applications of the particular test, - - - and - - quality control is needed to assure that the test results will satisfy the medical needs.”

*NCCLS C24-A2, Statistical Quality Control for Quantitative Measurements: Principles and Definitions.*

# Quality Control Requirements

- Contain all analytes being tested, including rare mutations NCCLS, CLIA
- Affordable and cost effective
- Monitors the entire testing process. “A control material must detect errors in the entire testing process.” CLIA Final Rule 2003.
- Stable
- Traceable

# Current Genetic Controls

Frozen DNA  
Cell lines

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## Issues

- Confidentiality and consent
- Limited available sources-lack of materials for rare mutations
- Variable stability-unable to validate lot-to-lot reliability
- Lack of convenience

# MMQCI Steps to Put the Genotype in the Bottle



1. DNA control sequences are synthesized
2. Mutations are created *in vitro*
3. Sequences are validated
4. Constructs are stabilized
5. Wild type & mutant constructs are combined to form genotypes

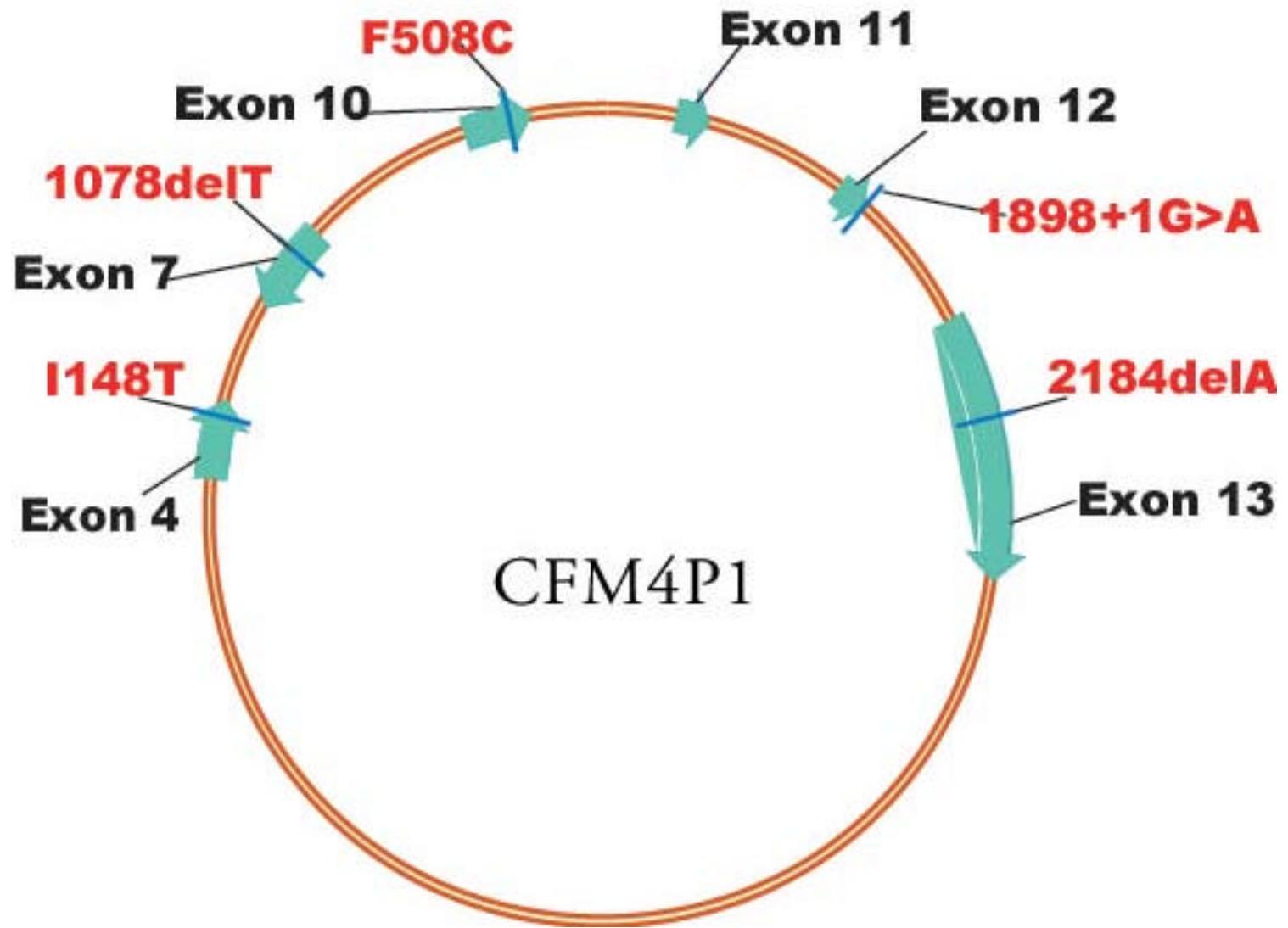
Patents Pending

# 1. DNA control sequences are synthesized

- Constructs are produced by high fidelity amplification, restriction digests, cloning
- Constructs have embedded restriction sites so new sequences can be added or exchanged

## 2. Mutations are created *in vitro*

- Mutagenesis by standard kit methods
- Several mutations in one control bottle = multiplex control for cost of one test



### **3. Sequences are validated**

- Validated by automated sequencing
- Traceability is ultimate target: “The traceability of values assigned to calibrators and/or control materials must be assured through available reference measurement procedures and/or available reference materials of a higher order.”

EU IVD Directive Annex 1 Sec 3.

## 4. Constructs are stabilized

- Processes: Fixed bacteria cells
  - Liposome complexes
  - Active surface particles
- Rigorously purified components
- Stability testing for shelf life:
  - Real time of 1 year
  - Accelerated of 5 years
- Extractable

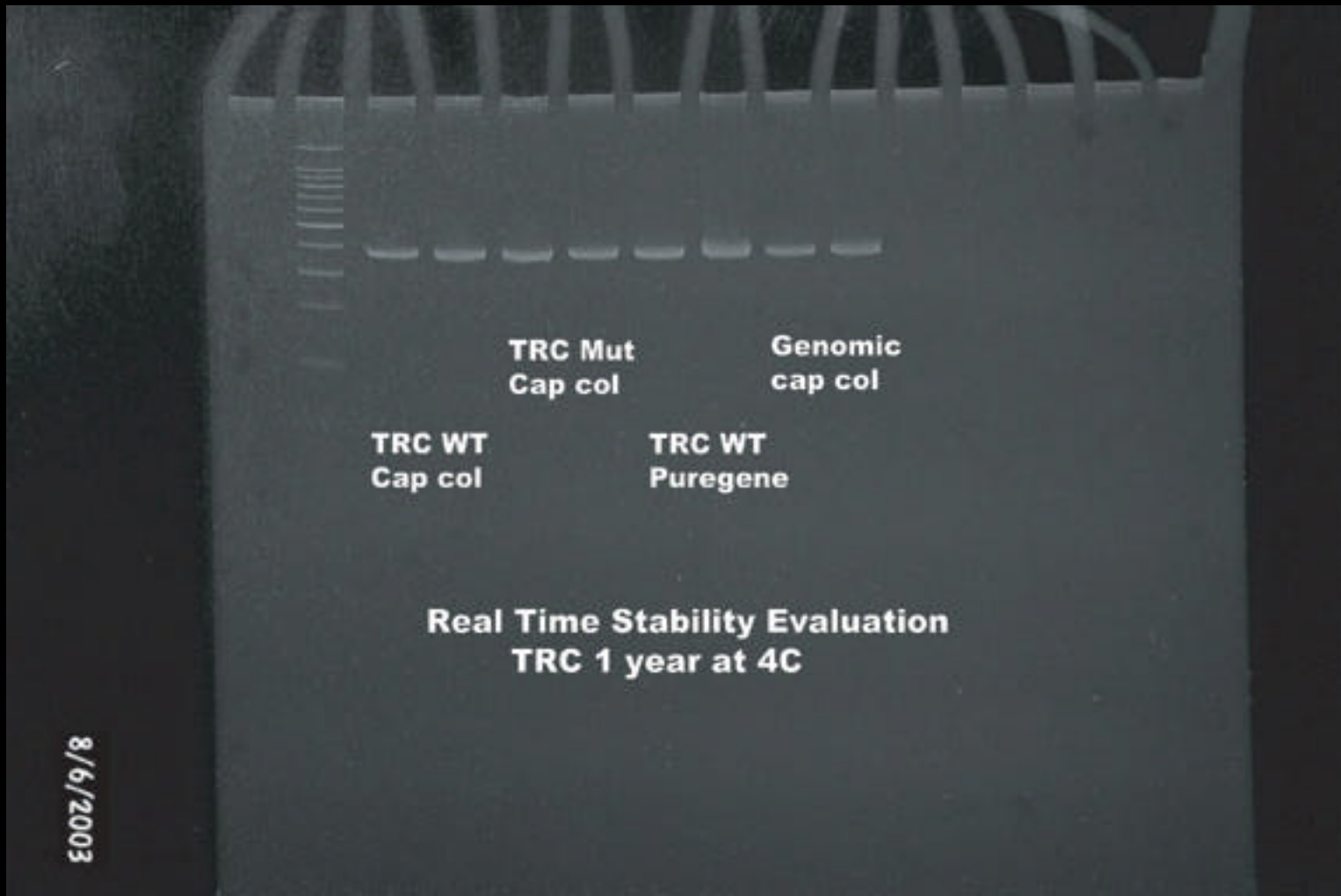
# **Thrombotic Risk Control (TRC) Real Time Stability at 4° C**

Roche LightCycler Prothrombin 20210

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	Crossing Point
Genomic	22.21 CP
TRC Baseline	20.28 CP
TRC 1 yr at 4C	19.40 CP

Samples extracted with Gentra Generation® Capture Column



**One year 4C stability - Prothrombin 20210 amplification**



## **Monitor the Entire Test System**

**“A control material must detect errors in the entire testing process.”**

**“For each test system, the laboratory is responsible for having control procedures that monitor the accuracy and precision of the complete analytical process.”**

**CLIA 88 Final Rule Feb. 24, 2003**

# **DNA Extraction Tests Performed:**

Gentra Puregene™

Gentra Generation™ (Column)

QIAGEN QIAamp™

Roche MagNA Pure™



**Prothrombin Test on Gentra Extractions  
Capture Column and Puregene of TRC**

## 5. Wild type & mutant constructs are combined

### Thrombotic Risk Control Panel

FV Leiden/ FII 20210

Vial 1	WT/ WT
Vial 2	Mut/ Mut
Vial 3	Hetero/ Hetero





# **CFM4P1 Control**

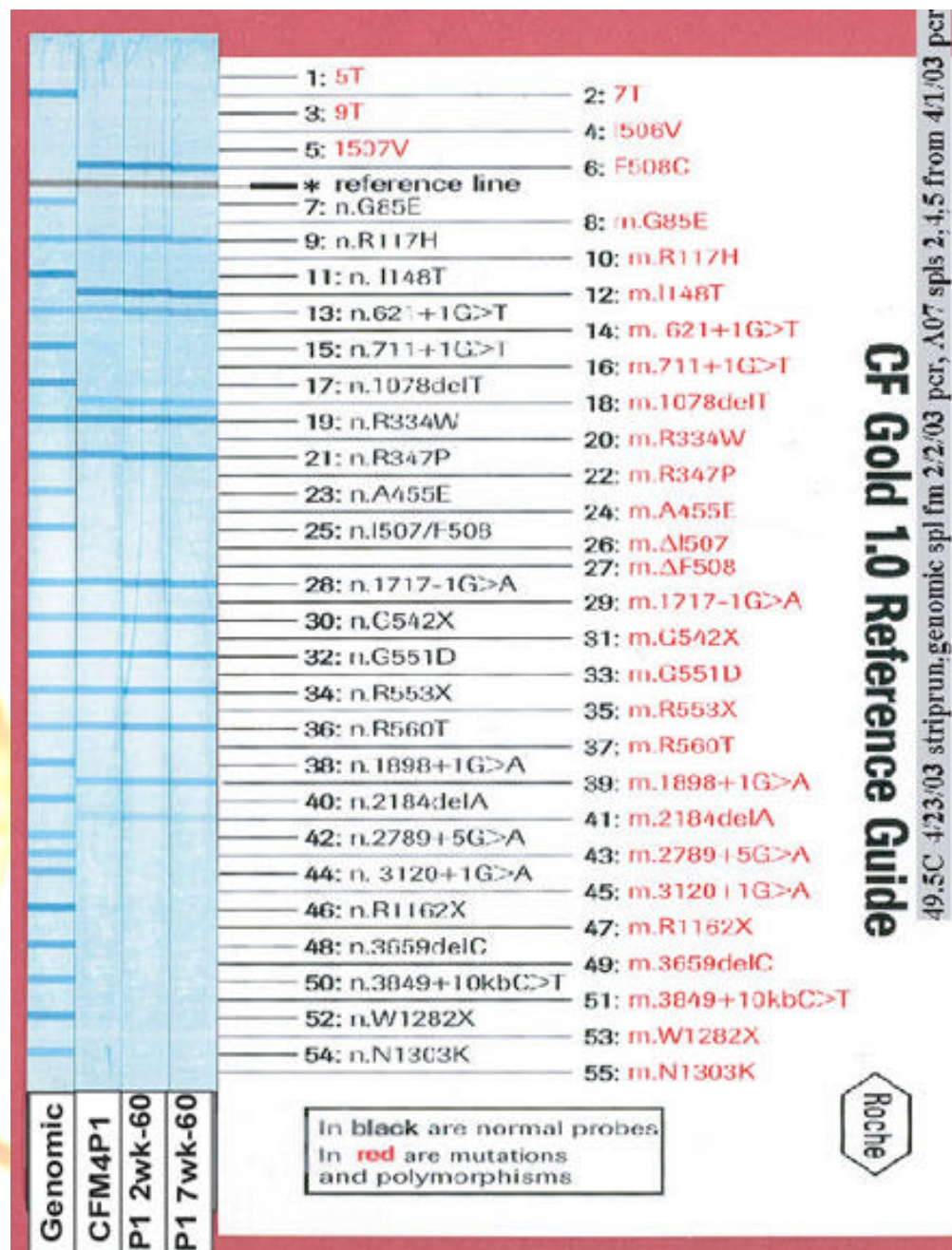
## **(One CF Test)**

**Exons:** 4, 7, 10, 11, 12, 13

**Mutations:** I148T, 1078delT, 1898+1G>A,  
2184delA

**Polymorphism:** F508C

**Systems tested:** Roche CF Gold 1.0, ABI/Celera  
CF ver 3.0 ASR, Nanochip® CFTR ASR,  
Elucigene™ CF 29



# The Future

- Test in wider variety of methods  
React to feedback/collaborate
- Obtain FDA approval
- Produce a quantitative control
- Adopt new technologies to synthesize constructs

# **Thank you for Support**

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MTI – Dr. Janet Yancey-Wrona



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